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Immunology

Inhibition of phosphatidylinositol 3-kinase activity by association with 14-3-3 proteins in T cells

(T-cell activation/tyrosine phosphorylation)

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ABSTRACT **Proteins of the 14-3-3 family can associate with, and/or modulate the activity of, several protooncogene and oncogene products and, thus, are implicated in regulation of signaling pathways. We report that 14-3-3 is associated with another important transducing enzyme, phosphatidylinositol 3-kinase (PI3-K). A recombinant 14-3-3 fusion protein bound several tyrosine-phosphorylated proteins from antigen receptor-stimulated T lymphocytes. PI3-K was identified by immunoblotting and enzymatic assays as one of the 14-3-3-binding proteins in resting or activated cells. Moreover, endogenous 14-3-3 and PI3-K were coimmunoprecipitated from intact T cells. Far-Western blots of gel-purified, immunoprecipitated PI3-K with a recombinant 14-3-3 fusion protein revealed direct binding of 14-3-3 to the catalytic subunit (p110) of PI3-K. Finally, anti-phosphotyrosine immunoprecipitates from activated, 14-3-3-overexpressing cells contained lower PI3-K enzymatic activity than similar immunoprecipitates from control cells. These findings suggest that association of 14-3-3 with PI3-K in hematopoietic (and possibly other) cells regulates the enzymatic activity of PI3-K during receptor-initiated signal transduction.**

Members of the 14-3-3 protein family form a group of highly conserved 27- to 30-kDa proteins that possess various biological activities, including activation of tyrosine and tryptophan hydroxylases; regulation of protein kinase C; stimulation of Ca^{2+}-dependent exocytosis; obligatory cofactor activity for ADP ribosylation by *Pseudomonas aeruginosa* exoenzyme S (denoted FAS activity); and regulation of gene transcription, development, and cell cycle (1–3). More recently, 14-3-3 proteins were found to directly bind several oncogene or protooncogene products that modulate signal transduction pathways—i.e., the Raf-1/B-Raf protein kinases (4–10), the Bcr or Bcr-Abl kinases (11), and the polyomavirus middle tumor antigen (12). Furthermore, activation of Raf-1 by 14-3-3 in intact cells or *in vitro* was also demonstrated in some (4–9), but not other (10), studies. On the basis of these findings, it was suggested that 14-3-3 proteins may regulate the function of signal-transducing protein complexes by modulating their enzymatic activity, conformation, stability, and/or intracellular localization (1–3).

The association of 14-3-3 proteins with molecules that are known to participate in signal transduction pathways and the fact that phosphorylation of proteins on tyrosine by receptor-coupled protein-tyrosine kinase (PTK) is a common event in such pathways raised the possibility that 14-3-3 proteins can associate, directly or indirectly, with additional signal-transducing proteins that may become tyrosine-phosphorylated upon cellular activation. In the course of characterizing such proteins by their *in vitro* binding to a recombinant 14-3-3 fusion protein, we identified phosphatidylinositol 3-kinase (PI3-K), an enzyme that plays an important role in PTK-coupled mitogenic and other signaling pathways (13, 14), as a 14-3-3-binding protein. Furthermore, we found that the catalytic subunit of PI3-K (p110) binds directly to 14-3-3 and that this association can reduce the enzymatic activity of tyrosine-phosphorylated PI3-K from activated T cells. These findings suggest that 14-3-3 can modulate signaling pathways that involve the action of PI3-K.

MATERIALS AND METHODS

Cell Stimulation and Lysis. Human Jurkat leukemia T cells were kept at logarithmic growth in RPMI 1640 medium supplemented with L-glutamine (2 mM), 5% heat-inactivated fetal calf serum, and antibiotics. Cells were harvested, washed, resuspended in 400 μl of phosphate-buffered saline (pH 7.2), and left unstimulated or stimulated for the indicated times at 37°C with OKT3 (10 μg/ml), an anti-human CD3 monoclonal antibody (mAb), or with 100 μM pervanadate. In some experiments, Jurkat cells were transiently transfected by electroporation with 10 μg of purified plasmid DNA from the pEFneo-14-3-3τ or empty pEFneo vector and grown for 2 days before stimulation and lysis. The *Eco*RI/*Xba* I insert from pME555 (see below) was cloned into the pEF mammalian expression vector (15). U-937 human monocytic leukemia cells were stably transfected with a pEF/14-3-3τ expression vector and selected by neomycin resistance. These cells express 3- to 4-fold higher than normal levels of 14-3-3. The cells were activated by crosslinking their surface Fc receptors for IgG with human IgG followed by a goat anti-human IgG antibody. Activation was terminated by adding 400 μl of 2× lysis buffer (40 mM Tris·HCl, pH 7.5/300 mM NaCl/10 mM EDTA/10 mM NaPP/10 mM NaF/4 mM Na_3VO_4/aprotinin and leupeptin each at 20 μg/ml). Lysates were mixed with recombinant 14-3-3 to assess binding or were subjected to immunoprecipitation (see below).

14-3-3 Binding. A cDNA clone (clone 555) encoding full-length human 14-3-3τ, an isoform encoded by a cDNA originally isolated from human T cells (16), was derived from a U-937 histiocytic lymphoma cDNA library. This cDNA was used as template to isolate by PCR the coding sequence of 14-3-3τ. The following primers were designed to introduce flanking 5′ *Eco*RI and 3′ *Xba* I restriction sites (underlined): 5′-CGAATTCGCCATGGAGAAGACTGAGCTG-3′ and 5′-GTCTAGATTAGTTTTCAGCCCCTTCTGC-3′, respectively. The 0.8-kb PCR-derived *Eco*RI/*Xba* I fragment was purified and ligated into the corresponding sites of pME18S

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Abbreviations: PI3-K, phosphatidylinositol 3-kinase; mAb, monoclonal antibody; PTK, protein-tyrosine kinase; GST, glutathione *S*-transferase.

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(17) to generate plasmid pME555. The integrity of the coding sequence was verified by sequencing. The *Eco*RI/*Xba* I fragment from pME555 was cloned into the glutathione *S*-transferase (GST) fusion vector pGEX-4T-2 (Pharmacia) by standard procedures. Expression of GST–14-3-3τ (or control GST) was induced with 0.5 mM isopropyl β-D-thiogalactopyranoside for 3 hr and the fusion protein was purified by using glutathione–Sepharose beads (Pharmacia). A GST fusion protein expressing the unique N-terminal, Src homology 2 and 3 domains of human p56^{lck} (Lck decoy) was prepared similarly. Cell lysates (10^7 cell equivalents) were incubated at 4°C for 2 hr with 10 μg of GST–14-3-3τ, GST–Lck decoy, or control GST protein, followed by 1 hr of incubation with glutathione–Sepharose beads. Bound proteins were washed extensively with 1× lysis buffer and analyzed by immunoblotting or PI3-K enzymatic assays.

Immunoprecipitation and Immunoblotting. The following antibodies were used for immunoprecipitation of cell lysates: Rabbit anti-PI3-K (p85) and anti-phosphotyrosine [Tyr(*P*)] mAbs were from Upstate Biotechnology (Lake Placid, NY); rabbit anti-PI3-K (p110α) or anti-Raf-1 and anti-GST mAbs were from Santa Cruz Biotechnology (Santa Cruz, CA). An anti-14-3-3 mAb was generated by immunizing mice with a recombinant 14-3-3τ protein. The antibody reacts with two 14-3-3 isoforms—i.e., τ and σ—of which only τ is expressed in T cells (H.Y., unpublished observations). A rabbit p110β-specific antiserum was generated by us by immunization with a synthetic peptide corresponding to the C terminus of p110β. Lysates were mixed with optimal concentrations of the respective antibodies for 2 hr, followed by addition of protein G-Sepharose beads for an additional hour at 4°C. Washed immunoprecipitates were collected by centrifugation, eluted into SDS sample buffer, resolved by SDS/8.5% PAGE, transferred to nitrocellulose, and immunoblotted with the indicated antibodies. Binding was detected with a horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin (ECL Western blotting detection system; Amersham, IL).

PI3-K Assay. Immunoprecipitated PI3-K was washed three times in lysis buffer, once in 20 mM Tris·HCl, pH 7.5/150 mM NaCl; once in 100 mM Tris·HCl, pH 7.5/0.5 mM LiCl; once in 20 mM Tris·HCl, pH 7.5/150 mM NaCl; and finally in 20 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM EDTA. PI3-K activity was measured as described (18). The assay mixture contained 20 mM Tris·HCl, pH 7.5/100 mM NaCl/0.5 mM EGTA/10 μg of phosphatidylinositol/20 mM $MgCl_2$/10 μCi of [γ-^{32}P]ATP (7000 Ci/mmol; 1 Ci = 37 GBq; Amersham). The reaction was stopped after 5 min at room temperature by adding 150 μl of chloroform/methanol/concentrated HCl (100:200:2) and the lipids were extracted after addition of 100 μl of chloroform. The organic phase was washed in methanol/1 M HCl (1:1) and analyzed by ascending chromatography on silica gel thin-layer chromatography plates in chloroform/methanol/25% ammonium hydroxide/water (90:90:9:19) followed by autoradiography. The position of phosphatidylinositol 3-phosphate was verified by including a ^{3}H-labeled standard inositolphospholipid.

RESULTS

Association of 14-3-3τ with Tyrosine-Phosphorylated T-Cell Proteins. To address the possibility that 14-3-3 associates, directly or indirectly, with Tyr(*P*)-containing proteins that may be involved in T-cell receptor/CD3-induced T-cell activation, we adsorbed lysates of resting or anti-CD3 (OKT3)-stimulated Jurkat cells on an immobilized, recombinant GST–14-3-3 fusion protein and probed bound complexes with an anti-Tyr(*P*) mAb. An additional group of cells was treated with pervanadate, which is known to induce tyrosine hyperphosphorylation and T-cell activation by inhibiting protein tyrosine phosphatases (19, 20). The 14-3-3 protein used was an isoform encoded by a cDNA originally isolated from human T cells (16), hereafter referred to as 14-3-3τ. A subset of tyrosine-phosphorylated proteins present in total lysates from anti-CD3- or pervanadate-treated and, to a much lower extent, from unstimulated Jurkat cells bound reproducibly to GST–14-3-3τ but not to the control GST protein (Fig. 1). The most prominent among these were ≈72- and ≈110-kDa proteins. Additional phosphoproteins of larger molecular mass and an ≈85-kDa protein also bound to GST–14-3-3τ. As expected, these proteins were more heavily phosphorylated on tyrosine in pervanadate-treated compared to anti-CD3-treated cells.

Direct Binding of PI3-K (p110) to 14-3-3τ. The sizes of the 14-3-3-associated 85- and 110-kDa proteins correspond to the regulatory and catalytic subunits, respectively, of PI3-K. Both subunits can be phosphorylated on tyrosine upon stimulation of different cell types (21–25). We ascertained, therefore, whether PI3-K can associate with 14-3-3τ. T-cell lysates were adsorbed on immobilized GST–14-3-3τ; bound proteins were separated by SDS/PAGE and immunoblotted with an anti-p85 antibody. The washed precipitates were assayed in parallel for PI3-K enzymatic activity. As a positive control for PI3-K binding under the same conditions, we used a GST fusion protein expressing an Lck decoy, which associates, like other Src family PTKs, with p85 via its Src homology 3 and/or 2 domains (26–29).

Immunoreactive p85 and PI3-K activity from both resting and anti-CD3-activated Jurkat cells bound to the 14-3-3τ and Lck decoy fusion proteins but not to the control GST protein (Fig. 2*A*). The matrix-bound protein recognized by the anti-p85 antibody comigrated with p85 present in total cell lysates. To further explore this interaction and its stoichiometry, we compared PI3-K levels in 14-3-3-bound complexes vs. those present in anti-p85 immunoprecipitates from the same cells. These experiments revealed that 10 μg of GST–14-3-3τ precipitated ≈1.5% of total PI3-K activity precipitated with anti-PI3-K antibodies (Fig. 2*B*). The GST–14-3-3τ-associated PI3-K activity was completely inhibited by 100 nM wortmannin (data not shown), a specific inhibitor of this enzyme (30). Although differences in the binding of PI3-K from resting vs. activated T cells to 14-3-3 were occasionally noted (e.g., Fig. 2*A*), these differences were not consistently detected, and their potential significance remains to be determined.

To determine whether the association between 14-3-3τ and PI3-K is direct, we probed membranes containing electrophoresed PI3-K immunoprecipitates with GST–14-3-3τ and detected binding with an anti-GST mAb. GST–14-3-3τ (but not

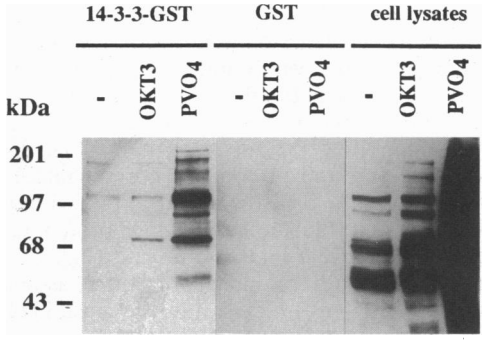

FIG. 1. Interaction of 14-3-3τ with Tyr(*P*)-containing proteins. Jurkat cells were left unstimulated (lanes −) or were stimulated for 5 min at 37°C with OKT3 (10 μg/ml) or pervanadate (PVO_4; 100 μM). Lysates (10^7 cell equivalents) were incubated for 2 hr with 10 μg of GST–14-3-3τ or control GST protein, followed by a 1-hr incubation with glutathione–Sepharose beads. Bound proteins were washed, resolved by SDS/8.5% PAGE, transferred to nitrocellulose, and immunoblotted with an anti-Tyr(*P*) mAb. Control anti-Tyr(*P*) immunoblots of total cell lysates (10^6 cell equivalents) are also shown. This experiment was repeated five times with similar results.

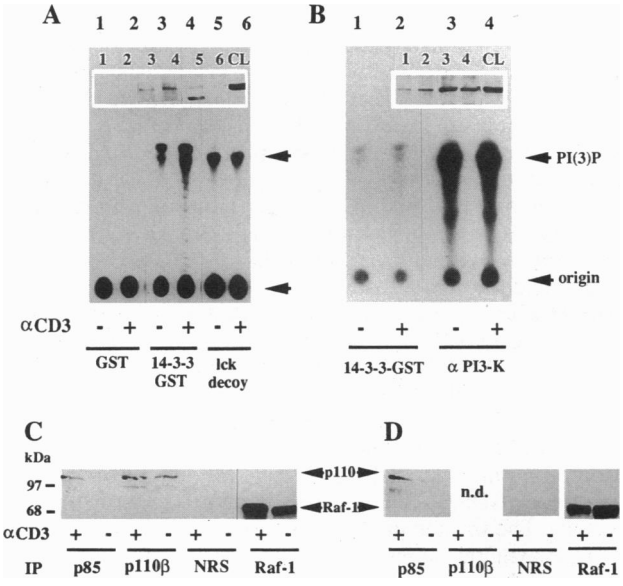

FIG. 2. Interaction of 14-3-3τ with PI3-K. (*A*) Lysates (10^7 cell equivalents) from unstimulated (lanes −) or 5-min OKT3-stimulated (lanes +) Jurkat cells were incubated with 10 μg each of purified GST–14-3-3τ, GST–Lck decoy, or control GST proteins, followed by glutathione–Sepharose beads. The washed beads were assayed for PI3-K activity. Parallel aliquots of the same precipitates or total cell lysate (CL; 10^6 cell equivalents) were resolved by SDS/8.5% PAGE, transferred to nitrocellulose, and immunoblotted with a PI3-K (p85)-specific antiserum (*Inset*). (*B*) PI3-K was immunoprecipitated from lysates of unstimulated (lanes −) or OKT3-stimulated (lanes +) Jurkat cells with an anti-p85 antiserum plus protein G-Sepharose beads. Immunoprecipitates and 14-3-3τ-bound proteins prepared as described in *A* were washed extensively and assayed for PI3-K activity. Parallel samples were resolved by SDS/8.5% PAGE and immunoblotted with a PI3-K-specific antiserum (*Inset*). This experiment is representative of three similar ones. PI(3)P, phosphatidylinositol 3-phosphate. (*C*) Normal rabbit serum (NRS), anti-PI3-K (p85 or p110β), or anti-Raf-1 immunoprecipitates (IP) from unstimulated or anti-CD3-stimulated Jurkat cells were separated by SDS/8.5% PAGE, transferred to nitrocellulose, treated with GST–14-3-3τ (10 μg/ml), and probed with an anti-GST mAb, followed by detection with a horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin. (*D*) The same membranes were stripped and reprobed with polyclonal anti-p110α (1:100; four left lanes) or anti-Raf-1 (1:500; two right lanes) antibody. The anti-p110β antibody was not adequate for immunoblotting; conversely, the anti-p110α antibody did not immunoprecipitate well. This experiment is representative of four similar ones. n.d., Not done.

the control GST protein; data not shown) bound to an ≈110-kDa protein that was immunoprecipitated by either anti-p85 or anti-p110β antibodies (Fig. 2*C*). Immunoblotting of the p85 immunoprecipitates with an anti-p110α antibody revealed a comigrating ≈110-kDa protein (Fig. 2*D*). This protein was not detected in control (normal rabbit serum) immunoprecipitates. As a positive control, 14-3-3τ also bound in similar far-Western blots to an ≈72-kDa protein comigrating with authentic Raf-1 from the same cells (Fig. 2 *C* and *D*). This is in agreement with the documented direct association of 14-3-3 with Raf-1 (4–10). Thus, 14-3-3τ most likely binds directly the catalytic subunit of PI3-K.

Association of PI3-K with 14-3-3τ in Intact T Cells. To address the physiological significance of the 14-3-3τ/PI3-K association, we ascertained whether endogenous p85 and 14-3-3τ interact at physiological concentrations in T cells. When 14-3-3 immunoprecipitates from resting or activated Jurkat cells were immunoblotted with an anti-p85 antibody, an immunoreactive band that comigrated with p85 present in the whole cell lysate was readily detected (Fig. 3*A Top Left*). Conversely, immunoblotting of p85 immunoprecipitates with

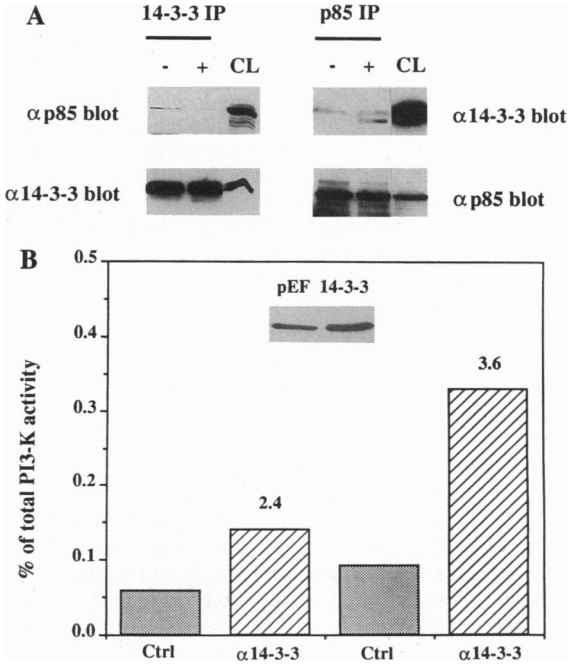

FIG. 3. Association of PI3-K with endogenous 14-3-3τ in intact T cells. (*A*) Lysates (10^7 cell equivalents) from unstimulated (lanes −) Jurkat cells or from cells stimulated for 5 min with OKT3 (lanes +) were immunoprecipitated (IP) with an anti-14-3-3 mAb or anti-PI3-K antiserum plus protein G-Sepharose as indicated. Washed immune complexes or total cell lysates (CL; 10^6 cell equivalents) were resolved by SDS/10% PAGE, transferred to nitrocellulose, and immunoblotted with the anti-PI3-K (*Top Left*) or anti-14-3-3 (*Top Right*) antibody. The lower ≈25-kDa band in the middle lane (lane +) (*Top Right*) represents the light chain of the stimulating OKT3 antibody. Samples were immunoblotted in parallel with the homologous antibodies to verify immunoprecipitation of the corresponding proteins—i.e., 14-3-3τ (*Bottom Left*) or p85 (*Bottom Right*). A similar coimmunoprecipitation was observed in four independent experiments. (*B*) Jurkat cells were transiently transfected by electroporation with 10 μg of purified plasmid DNA from the pEFneo-14-3-3τ or empty pEFneo vector and grown for 2 days. Cells (10×10^6) were left unstimulated or were stimulated for 5 min with OKT3, lysed, and immunoprecipitated with the anti-14-3-3 mAb or with an isotype-matched control (anti-GST; Ctrl) mAb, followed by protein G-Sepharose. Immunoprecipitates were washed extensively and assayed for PI3-K activity. Activity is expressed as percentage total anti-p85 precipitable activity (150,690 ± 11,600 cpm incorporated into phosphatidylinositol 3-phosphate in duplicate samples). Numbers above bars denote -fold increase in PI3-K activity in the specific immunoprecipitate relative to the control immunoprecipitate. Expression levels of 14-3-3τ in the transfected cells were determined by SDS/10% PAGE separation of cell lysates (10^6 cell equivalents) and immunoblotting with an anti-14-3-3τ mAb (*Inset*).

the anti-14-3-3 mAb revealed an ≈29-kDa band in the same position as that present in a whole cell lysate (Fig. 3*A Top Right*). Immunoblotting with the homologous antibodies confirmed the presence of 14-3-3τ or p85 in the corresponding immunoprecipitates (Fig. 3*A Bottom*).

The p85/14-3-3τ association was confirmed by assaying 14-3-3 immunoprecipitates from Jurkat cells, which were transiently transfected with a control vector or with a 14-3-3τ expression vector, for PI3-K activity. The specific immunoprecipitates contained 2.4- to 3.6-fold higher PI3-K activity than was present in those prepared with an unrelated, isotype-matched mAb (Fig. 3*B*). Consistent with the higher expression level of 14-3-3τ in the transfected cells, the 14-3-3τ-associated PI3-K activity in these cells was correspondingly higher. Based on a comparison between the coprecipitated vs. total precipitable levels of PI3-K and 14-3-3 present in the cells, we estimate that 0.2–0.5% and ≈1% of total PI3-K and 14-3-3τ,

respectively, coprecipitate from intact Jurkat cells under the conditions we used. In two of four experiments, we were also able to detect in p85 immunoprecipitates from Jurkat cells low but significant FAS activity (31) measured by exoenzyme S-mediated ADP ribosylation of bovine serum albumin (data not shown).

Modulation of PI3-K Activity by 14-3-3τ. The physiological role and functional consequences of the association between 14-3-3 proteins and different signaling molecules are far from clear. To address this issue with regard to the PI3-K/14-3-3τ association, we prepared anti-Tyr(*P*) immunoprecipitates from Jurkat cells transiently transfected with a control vector or with a 14-3-3τ expression vector and assayed them for PI3-K enzymatic activity. The use of such immunoprecipitates as a source of PI3-K activity reflects the activated fraction of PI3-K that participates in the signaling process upon cellular activation.

As expected, the levels of PI3-K activity (Fig. 4*A*) and immunoreactive p85 (Fig. 4*B*) were markedly increased in the anti-Tyr(*P*) immunoprecipitates from anti-CD3-activated Jurkat cells by comparison with the nonactivated cells, reflecting the activation-induced phosphorylation of PI3-K itself and/or associated proteins. More importantly, overexpression of 14-3-3τ in the cells reduced by ≈50% the enzymatic activity of PI3-K in the anti-Tyr(*P*) immunoprecipitates from anti-CD3-activated Jurkat cells (Fig. 4*A*) without affecting the amount of immunoreactive p85 present in the same immunoprecipitates (Fig. 4*B*). The low basal activity present in immunoprecipitates from nonactivated cells was not detectably reduced. Under these conditions, there was no detectable reduction in tyrosine phosphorylation of p110 detected by anti-Tyr(*P*) immunoblotting (data not shown), indicating that 14-3-3 does not decrease the anti-CD3-induced tyrosine phosphorylation of PI3-K.

DISCUSSION

The present results add PI3-K, a critical enzyme in growth and differentiation signaling pathways (13, 14), to the growing list of oncogene or protooncogene products that have been found to associate with members of the 14-3-3 protein family. Since PI3-K is known to associate with the polyomavirus middle tumor antigen (32), it is possible that PI3-K contributes to the recently reported association of the latter with 14-3-3 proteins in insect cells (12). Although the stoichiometry of the 14-3-3τ association with p85 is low (Fig. 3), it was of the same order of magnitude as that of the 14-3-3τ/Raf-1 association in the same cells (data not shown) and, thus, may be biologically significant. Furthermore, the lysis conditions used may be unfavorable for maintaining the endogenous PI3-K/14-3-3 association and, thus, the stoichiometry of this association in intact cells may, in fact, be significantly higher.

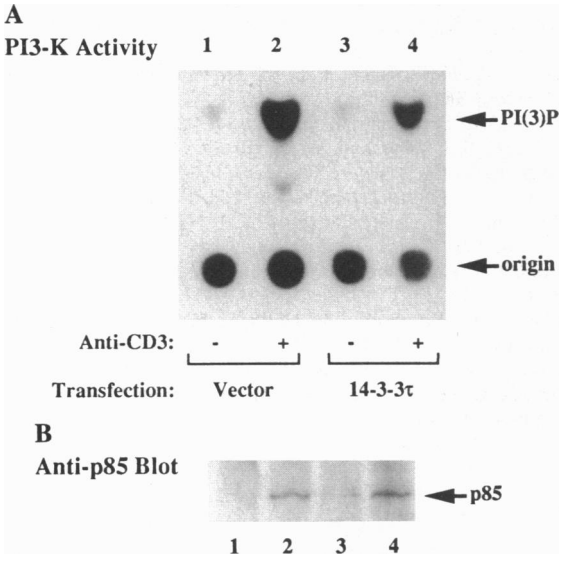

FIG. 4. Inhibition of PI3-K activity in anti-Tyr(*P*) immunoprecipitates from 14-3-3τ-overexpressing cells. Jurkat cells were transiently transfected with empty pEFneo or pEFneo-14-3-3τ vectors. Forty-eight hours later, anti-Tyr(*P*) immunoprecipitates (10^7 cell equivalents) were prepared with the 4G10 mAb from unstimulated cells or from cells stimulated for 5 min with anti-CD3 antibody and assayed for PI3-K activity. Aliquots of the same immunoprecipitates (5×10^6 cell equivalents) were immunoblotted in parallel with a PI3-K (p85)-specific antiserum. Similar results were obtained in three different experiments, each performed on duplicate samples. PI(3)P, phosphatidylinositol 3-phosphate.

Of particular interest is the finding that overexpression of 14-3-3τ in Jurkat T cells by transient transfection resulted in a consistent, ≥50% reduction in the amount of PI3-K activity precipitated from activated cells by an anti-Tyr(*P*) mAb. Since PI3-K activity was derived from all the cells, whereas the transfected 14-3-3τ was expressed in only a fraction of cells, this result represents, most likely, an underestimate of the real inhibitory effect of 14-3-3 on PI3-K activity. Similar reduction in PI3-K activity was observed in stably 14-3-3τ-transfected U-937 human monocytic leukemia cells, which were activated by crosslinking their Fc receptors for IgG (data not shown). The findings that the level of anti-CD3-induced tyrosine phosphorylation of PI3-K and the amount of immunoprecipitated PI3-K protein were not reduced under the same conditions indicate that the reduction in activity reflects direct inhibition of enzymatic activity.

Proteins of the 14-3-3 family were found to display a wide range of apparently unrelated biological activities (1–3, 31). The physiological role of 14-3-3 proteins and their structure–function relationship are still poorly understood, and it is unknown whether distinct members of the mammalian 14-3-3 family differ in their biological activities or interactions with other proteins. Furthermore, apparently contradictory results were reported describing the ability of 14-3-3 to either inhibit (33) or stimulate (34) protein kinase C activity. Similarly, whereas several studies reported activation of Raf-1/B-Raf kinases by 14-3-3 (4–9), the recent finding that mutant Raf-1 proteins unable to stably interact with 14-3-3 display enhanced enzymatic activity (10) implies a negative regulatory role for 14-3-3. It has been reported that 14-3-3 binds phosphorylated but not unphosphorylated forms of Raf-1 (10) and tryptophan hydroxylase (35), suggesting that binding to phosphorylated residues may be a common mechanism whereby 14-3-3 interacts with other proteins. However, a unifying model that accounts for their diverse activities is still missing.

A recently evolving concept implicates molecular chaperones, proteins that assist protein folding in general, as important regulators of signal transduction pathways by different receptor types, including receptor PTKs (36, 37). Genetic approaches have identified several chaperones, such as Hsp90, as being essential for various signaling responses. It has been suggested that the association of a given signaling protein with its corresponding chaperone is important for establishing and maintaining a poised conformation of the signal transducer that is optimally receptive to an incoming activating signal and for proper folding after ligand binding (36, 37). A recent study demonstrated opposite effects of mutations in one chaperone, yeast YDJ1, on its different targets—i.e., activation of the glucocorticoid and estrogen receptors and, conversely, inhibition of the $p60^{v\text{-}src}$ PTK (38). This is reminiscent of the apparently contradictory effects of 14-3-3 proteins on some of their targets. We would therefore like to propose that 14-3-3 proteins represent a distinct family of molecular chaperones, perhaps possessing a relatively limited substrate specificity (e.g., for Raf-1, Bcr, PI3-K, and protein kinase C), that regulate the activity state of these critical signal-transducing elements by associating with them. Such a model could ac-

count for the diverse associations and biological activities of 14-3-3 proteins.

14-3-3 proteins were recently found to activate Raf-1 (4–9), an important Ras effector that associates directly with the active, GTP-bound form of Ras (39–43), thereby leading to activation of a downstream serine/threonine kinase cascade (44, 45), including in T cells (46). In addition, active Ras was also recently found to associate with the catalytic subunit of PI3-K and stimulate its enzymatic activity (47, 48), indicating that PI3-K is another downstream effector of Ras. Thus, although the functional significance of the association between 14-3-3 and PI3-K remains to be determined, our results suggest that 14-3-3 proteins may regulate divergent Ras-dependent signaling pathways by associating with at least two Ras effectors.

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